the equilibrium is far in the direction of the starting complex (Eq. 2). The two isostructural Ir and Rh complexes usually appear to undergo analogous reversible addition reactions, but [RhCl(CO)(Ph_3P)$_2$] is considerably more stable (*15*) which renders its adduct often undetectable (*13*).
15. L. Vaska and S. S. Bath, *J. Am. Chem. Soc.* **88**, 1333 (1966).
16. On the basis of two commonly encountered configurations there are eleven possible geometrical isomers of the five-coordinated complex: five isomers are based on trigonal bipyramidal and six on tetragonal pyramidal configurations. Of the 11 possible isomers, there are five isomers in which the two CO ligands are equivalent, three in which the two Ph_3P groups are *trans* to one another, and two structures in which both of these conditions are met: (i) a *trans*-(Ph_3P)$_2$—*trans*-(CO)$_2$ tetragonal pyramid with Cl at the apex and (ii) the configuration in Fig. 1*C*. The formation of the latter from the starting complex (Fig. 1*A*) involves less structural change than the formation of (i).
17. According to the evidence presented here, the two carbon monoxide ligands in the CO adduct appear to occupy equivalent positions. Hence, the formula of the adduct should be written as [IrCl(CO)$_2$(Ph_3P)$_2$]. The alternative expression which is used in text and in Table 1 emphasizes the reversibility of the carbonylation reaction (Eq. 2 and Fig. 1), and its use is thus appropriate in the context of this report.
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21. This assignment is uncertain at present because of the low intensity of the band and because it occurs near the lower end of the available frequency range of the spectrophotometer used (200 cm^{-1}, Beckman IR-12). It is certain, however, that the original ν_{IrCl} in the spectrum of the parent compound (Table 1) is absent in the spectrum of the CO adduct, and that it has probably shifted to a lower frequency, in agreement with the increase of the coordination number of iridium from four to five.
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21 March 1966

Osmotic Pressure Influence in Germination Tests for Antibiosis

Abstract. *A common test for the presence of toxic organic substances in plant tissues, and therefore of the potential role of antibiosis (specifically allelopathy) in native plant communities, has been to apply a water extract of the tissues in germination tests of cultivated annuals. The observed osmotic pressure of the extracts can be high, and sucrose solutions of similar osmotic pressure result in a depression of germination and early development. Thus in the aforementioned type of test, extracts must be diluted to an osmotic pressure no greater than 0.5 atmospheres.*

The significance of chemical competition between higher plants, allelopathy, has been debated for many years (*1, 2*). The earliest studies were directed toward relieving the apparent "soil sickness" that followed cultivation of certain crops such as citrus fruits. Borner (*2*) concluded that the soil deterioration phenomenon was insufficiently investigated but that there was enough evidence to show that some soils could be made poisonous to other plants by organic compounds originating with a preceding plant cover. He noted also that in tests for the occurrence of toxic compounds, attention should be directed to their identification, as well as to the study of their physiological effects upon other plants or associated microorganisms.

The difficulty in isolating chemical constituents of water extracts from parts of plants growing both above and below ground has restricted study to a few of the most obvious examples (*3–5*) with readily distinguished toxic chemicals. More rapid survey techniques have been sought by investigators concerned with the occurrence of allelopathy among native plants, whether for pharmacological and medical studies (*6*), or as a factor in the distribution of plants in native vegetation (*7*). These methods have ranged from direct use of plant tissue remains, suspected of containing a toxic material, to application of a water extract from the plant parts. The plant materials or extracts usually are applied directly to potted plants or germinating seedlings (*4, 5, 8, 9*). In tests of germination and early seedling development, the responses are clear and readily measurable. With the wider acceptance of allelopathy as a fact, there have been increasing data purporting to show toxic materials in the water extracts from many native species.

Only a few of these papers consider that a moderate osmotic pressure in the extract could repress growth and germination through a direct influence on water uptake (*10, 11*). In general, either this influence has been ignored, or the assumption is made that since the materials are water extracts, there will be rapid build-up in osmotic value within the cell, and therefore normal uptake of water. Koller (*10*) found that a water extract of *Atriplex* fruit bracts had an osmotic pressure of 6.31 atm, but concluded that his data did not eliminate the possibility of an allelopathic substance being present. Torres *et al.* concluded that osmotic pressure was not a factor in germination inhibition by *Zinnia*. However, the osmotic concentration of their *Zinnia* extract was less than one atm.

In most of these studies the retarding of germination, and the depression of seedling growth, has been assessed by comparison with control seedlings cultured in distilled water. Since the object of the tests has been to demonstrate and measure the strength of allelopathic effects, a more suitable control would be a nontoxic substance, such as sucrose, giving osmotic concentrations in the same range as those of the diluted extracts. In this study a 0.05*M* sucrose solution in a simple columnar osmometer supported a column of water equal in height to that supported by a 1:10 extract of *Antennaria*. The osmotic pressures of the two solutions were 1:3 atm (*12*) and 2.4 atm respectively. The sucrose solutions were therefore adopted as controls against which to measure the degree of inhibition produced by the plant extracts. Additional controls cultured with distilled water were used as necessary.

Materials from five species of plants were used to prepare the plant extracts. *Lonicera tatarica* leaf tissue, whole *Antennaria neglecta* plants, and the rhizomes of *Aster macrophyllus, Pteridium aquilinum* and *Helianthus laetiflorus* were oven-dried and ground, and the water-soluble materials were extracted. Production of allelopathic substances had been suggested for *Aster, Antennaria, Helianthus*, and *Pteridium* (*3, 9*); *Lonicera* was suspected of producing a toxic substance because of its ability to invade native Wisconsin plant communities.

In the preparation of the extracts, ten parts by weight of hot, double-distilled water were poured into a beaker containing 1 part of plant tissue. The material was stirred for 10 minutes and then strained with light manual pressure through two layers of cheese

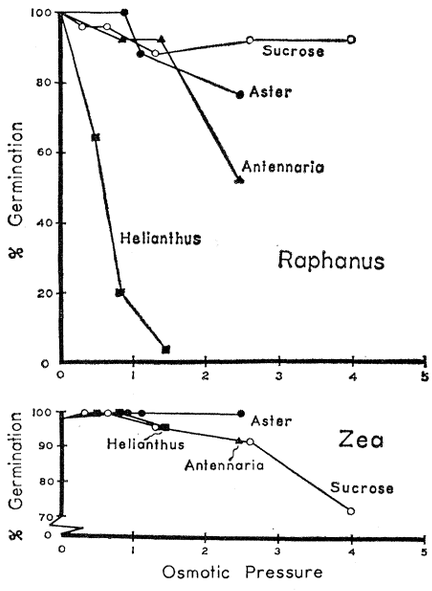

Fig. 1. The effect of a range in osmotic pressure of sucrose solutions and three plant extracts on the germination of corn (*Zea*) and radish (*Raphanus*).

cloth into a Buchner funnel. The volume of water extract recovered varied from 61 to 71 percent of the total volume of water added. This solution was used as the 1:10 concentration, and dilutions of the extract to 1:20 and 1:40 were made from it.

The freezing point of each extract and of each dilution was obtained by means of a Beckman cryoscopic apparatus. Three readings of the observed freezing point were obtained on each sample, three-thousandths variance in replication being tolerated. The osmotic pressure was computed by the Harris and Gortner procedure (*13*). The results (Table 1) show a threefold range in osmotic pressures of the 1:10 extracts. Even with the 1:40 dilution

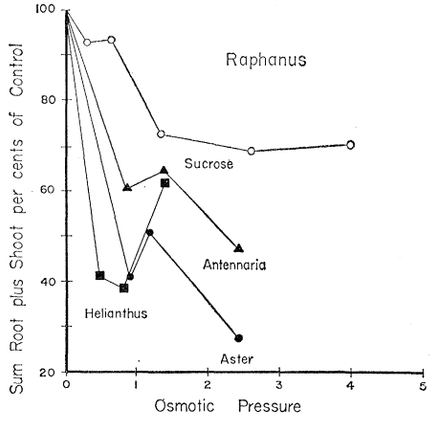

Fig. 2. The effect of a range in osmotic pressure on the growth of germinating radish (*Raphanus*).

commonly used in testing plant extracts, the threefold range in osmotic pressure remains. The highest values at 1:10 and 1:20 dilutions are well within the range of osmotic pressures considered effective in reducing water uptake (*14*).

The plant extracts and a series of dilute sucrose solutions ranging in osmotic pressure from 0.3 to 4.0 atm were applied to seeds of radish, corn, and barley to test germination and seedling development. Twenty-five seeds of each species were placed in sterile glass petri dishes (9 cm) containing a single layer of Whatman No. 2 filter paper. Small additions of distilled water kept the filter paper moist. For radish and barley the treatment and observations were terminated after 3 days; corn required 7 days to reach a comparable stage in seedling development.

In each plate the number of germinating seeds was counted. The percentage germination of corn and radish in the four sucrose solutions and three concentrations of *Antennaria, Helianthus*, and *Aster* extracts is shown in Fig. 1. The effect of the *Helianthus* on radish germination is clearly distinguished from that of the other extracts, even at low osmotic pressure. The only other extract that retarded radish germination appreciably was that of *Lonicera*, but it was effective only at high osmotic pressures. In contrast, none of the extracts inhibited the germination of either corn or barley, the other monocot tested. In general, the responses of corn and barley form a gradient corresponding to the increases in osmotic concentration.

The length of both root and shoot development of each seedling was measured to obtain the average root and average shoot for each of the treatments. The average for each treatment is expressed as a percentage of the growth in the water-control group. Analysis showed that the treatments affected both root development and shoot growth equally. The sum of these two percentages divided by two therefore shows the combined inhibition of roots and shoots. The responses of radish to four of the treatments are plotted in Fig. 2.

The results in Figs. 1 and 2 show marked diversity in seed germination and seedling development, depending on the treatment (plant extract compared to sucrose) and the measure of response. The extracts produced a considerable reduction in the germination of radish compared to corn, but only *Helianthus* was effective at low osmotic

Table 1. Cryoscopic determinations of osmotic pressure at three dilutions of water extracts.

Plant	Extract dilution		
	1:10	1:20	1:40
Lonicera tatarica	4.52	2.19	1.56
Aster macrophyllus	2.46	1.10	0.89
Antennaria neglecta	2.42	1.39	.86
Pteridium aquilinum	2.41	1.08	.69
Helianthus laetiflorus	1.44	0.81	.49

pressures. On the other hand, *Helianthus* was not consistently effective in reducing elongation of radish seedlings. *Aster* appears to be as effective as *Helianthus* in retarding growth of radish, but it was not effective in repressing germination at low osmotic pressures.

With one exception, all of the extract concentrations examined inhibited the growth of radish seedlings more than sucrose solutions of comparable osmotic pressures. However, none of the extracts was appreciably more effective in retarding growth and germination in corn and barley than were sucrose solutions of comparable osmotic pressures. The lowest concentrations of sucrose (0.01 and 0.025*M*; 0.3 and 0.7 atm) actually stimulated the growth of corn and barley (*14*). At higher concentrations the retarding effect of sugar on growth closely approached that of the plant extracts. The growth and germination of radish seedlings was depressed by all concentrations of sucrose but the greatest inhibition occurred as the concentration of sucrose was increased from 0.3 to 1.3 atm. Additional increases in sucrose concentration from 1.3 to 4.0 atm did not produce any additional reduction in germination or growth.

Thus major improvements in technique can be made in the search among higher plants for toxic substances which may be effective in medicine, weed control, and in studies of native plant communities. When the allelopathic potential of a water extract is being assessed, the inhibition attributable to the osmotic influence of the extract must be considered. Our results show that osmotic pressure is an important factor in allelopathic tests. Repression of growth by extracts of unknown and possibly high osmotic concentrations cannot be taken to indicate the presence of toxic substances.

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11 March 1966

Immunochemical Characterization of Polyribonucleotides

Abstract. *The degree of organization of polyribonucleotides determines their modalities of reaction with antibodies (NG-I) which are present in serums of animals hyperimmunized with ribosomes. The immunochemical behavior of the highly helical two-stranded complex of polyadenylic acid and polyuridylic acid and the corresponding three-stranded complex of one molecule of polyadenylic acid and two molecules of polyuridylic acid can be determined from examination of the associating and nonassociating mixtures of polyadenylic and polyuridylic acids. The immunochemical characteristics of various forms of polyinosinic acid in solution are described.*

RNA (*1*), DNA (*1*, *2*), and synthetic polynucleotides (*3*) are not immunogenic. However, the presence of antibodies active against denatured DNA has been demonstrated in the serums of patients with systemic lupus erythematosus (*4*). The serums of rabbits immunized with ruptured T-even coliphages similarly contain antibodies reacting with denatured DNA, which are directed, in part, either toward the α-gentiobioside residue of DNA, or toward the α- or β-monoglucosylhydroxymethylcytosine component (*5*).

The antibodies prepared by the procedure of Plescia *et al.* (*5*) had specificities similar to those produced when ruptured bacteriophage was used as the antigen. In addition, various authors (*6*) have investigated the specificity of antibodies resulting from immunization with synthetic agents such that a purine or pyrimidine base was conjugated with a protein antigen. However, even when uridine was conjugated, the resultant antibodies reacted indifferently with heat-denatured DNA and RNA, but not with double-stranded DNA or native RNA.

Antibodies from the serums of animals hyperimmunized with ribosomes (*7*) differ markedly from the above-mentioned antibodies. In the first place, they are produced under very different immunogenic conditions. Second, certain of these antibodies—to which we refer as "NG-I" antibodies, and which we have isolated and purified from horse serums by two or three specific precipitations with polyA (*8*)—precipitate native RNA (soluble, ribosomal, and viral) and various polyribonucleotides, although they still remain inactive with respect to native and denatured DNA.

Furthermore, the immune reaction with NG-I is not inhibited by purine or pyrimidine bases, or by ribo- or deoxyribonucleosides, or by mono-, di-, or trinucleotides (*7*). The foregoing results signify that at least a part of the antigenic determinant is common to all ribonucleic acids and synthetic polyribonucleotides.

We now report on how the extent of organization of polyribonucleotide determines its immunochemical behavior with relation to these NG-I antibodies. First of all, we studied the precipitation near neutrality of polyU and of polyA (*8*) with NG-I antibodies in the absence of electrolytes as well as in the presence of $5 \times 10^{-4}M$ Mg^{++} (Fig. 1, *a* and *b*). Since polyU is randomly coiled under normal conditions, as shown by the failure of the physicochemical investigations to obtain any indication of ordered structure (*9*), the precipitation of antibody and of antigen can be taken as the norms of the immunochemical reaction of an unordered polyribonucleotide (*10*).

The curves of precipitation of polyA near neutrality are identical, up to the zone of equivalence, to those plotted for polyU. This shows (i) that the nature of the base is not a quantitative determinant in the reaction, and (ii) that, under these conditions of ionic strength and *p*H, polyA does not have intermittent ordered regions, which would have decreased the amount of antibody precipitated (*11*).

In the presence of excess antigen, inhibition of the precipitation of polyA is less than that noted under the same conditions for polyU. This is probably related to the lower molecular weight of polyU as compared with that of polyA (*12*). Kabat (*13*) observed the same phenomenon in the case of dextrans of varying molecular weight.

The following experiments show that the immunochemical behavior of highly helical polyribonucleotide complexes such as poly(A+U) and poly(A+2U) (*14*) differs greatly from that of unorganized homopolymers. The immunochemical behavior of these complexes can only be appreciated with reference to the behavior of a corresponding non-

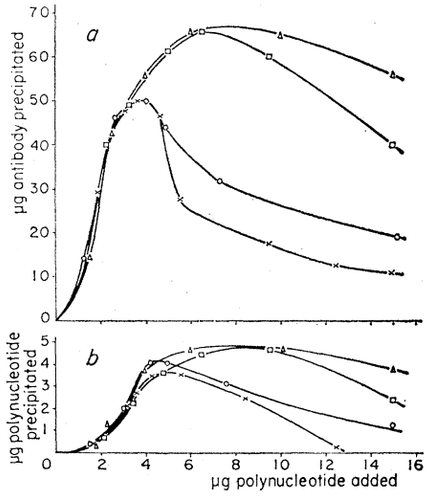

Fig. 1. Amounts of antibody (*a*) and polynucleotide (*b*) precipitated by polyU and polyA ("neutral form"). The polyribonucleotides (Miles) were dialyzed against $3 \times 10^{-3}M$ EDTA and then thrice-distilled water. Stock solutions of antibodies and polynucleotides, stored at −20°C in the absence of electrolytes, were diluted in thrice-distilled water and adjusted to *p*H 7.4, and $5 \times 10^{-4}M$ Mg^{++} when indicated. The polyribonucleotides were added in ever-increasing amounts on a mononucleotidic basis, the amount of antibody being kept constant (110 µg). The specific precipitates were collected, washed three times in ten volumes of $0.1M$ NaCl, $0.0033M$ Mg^{++} acetate, $0.0017M$ tris buffer, *p*H 7.4, and then dissolved in 0.3 ml of $0.1N$ NaOH containing 1 percent Na_2CO_3. One portion of the specific precipitate was used for estimating the proteins (*18*). The other served for the estimation of nucleotides: optical density measurements were made at 260 mµ for polyU and at 257 mµ for polyA, after hydrolysis by $1N$ perchloric acid. ×———×, PolyU in absence of electrolytes; ○———○, polyA in absence of electrolytes; □———□, polyU in $5 \times 10^{-4}M$ Mg^{++}; △———△, polyA in $5 \times 10^{-4}M$ Mg^{++}.